

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Continuation-in-part

Patent Application for

Microfluidics Apparatus and Methods for Use Thereof

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Microfluidics Apparatus and Methods of Use Therefor

5 The present application is a continuation-in-part of the application entitled
"Microfluidics Apparatus and Methods of Use Therefor" bearing Attorney
Docket 2506/129 which was filed in the United States Patent and Trademark
Office on November 9, 2001, and which is incorporated herein by reference.

Technical Field

10 The present invention relates to chemical analysis, and in particular to
methods and apparatuses for performing chemical analysis of biomaterials with a
microfluidics device using microcantilevers.

Background of the Invention

15 It is known that thin bimorph microcantilevers can undergo bending
(deflection) due to differential stresses following exposure to and binding of a
compound from their environment, for example in a fluid sample. Soft
microcantilevers having spring constants less than 0.1 N/m are sensitive to stress
20 differentials that arise as a result of interactions between extremely small
amounts of a substrate material on a surface of the microcantilever and one or
more materials in a sample. For a given microcantilever with a specially
designed coating layer, the deflection yields information about components of
the environment to which the microcantilever is exposed.

Microcantilevers are capable of detecting calorimetric enzyme-mediated
25 catalytic biological reactions with femtoJoule resolution. (Thundat et al.,
"Microcantilever Sensors", Microscale Thermophysical Engr. 1, pgs. 185-199,
1997.) Further, oligonucleotide interactions within a sample can be detected
using a monolithic array of test sites formed on a surface to which the sample is
applied as shown in U.S. Patent No. 5,653,939.

30 It is also known to provide integrated chips to categorize molecules in a
biochemical sample. For example, U.S. Patent 6,123,819 to Peeters discloses a

design for an integrated chip having an array of electrodes at the atomic or nano scale. The chip can be used to characterize single molecules in a solution such as individual proteins, complex protein mixtures, DNA, or other molecules.

In recent years, microfluidics technology employing microcantilevers has emerged to provide a "lab-on-a-chip" for chemical analysis of biomaterials. For example, U.S. Patent No. 6,054,277 to Furcht et al. discloses a genetic testing system that includes an integrated, unitary microchip-based detection device with microfluidic controls. The device employs a microcantilever sensor to detect a biochemical reaction in a single detection chamber having capillary interconnects. However, to analyze a number of solutions simultaneously, it would be necessary to utilize an equal number of these chips

Summary of the Invention

In one embodiment, a microfluidics device is provided. The microfluidics device comprises a plurality of interaction cells and fluid control means, including: i) means for providing to the interaction cells a preparation fluid, and ii) means for providing to the interaction cells a sample fluid, wherein each interaction cell receives a different sample fluid. In a related embodiment, a plurality of microcantilevers is disposed in each of the interaction cells, each of the plurality of microcantilevers being configured to deflect in response to an interaction involving a component of the sample fluid. The fluid control means may include means for removing a fluid from the interaction cells. The fluid control means may be robotic or it may be manual. Additionally, the plurality of microcantilevers may be provided as a plurality of fingers in a planar array. In accordance with another related embodiment, the microfluidics device is disposable. In another related embodiment, the microfluidics device is reusable.

In accordance with another embodiment, a microcantilever platform includes a plurality of interaction cells, each of the interaction cells including an inlet for receiving a sample fluid, wherein each of the interaction cells receives a different sample fluid; at least one microcantilever is disposed in each of the interaction cells, the microcantilever being capable of deflecting in response to

chemical interaction with a component of the sample fluid. In related embodiments, each interaction cell further includes at least one outlet whereby fluid may flow out of the cell. The microcantilever platform may be disposable or reusable.

5 In accordance with a further embodiment, an apparatus is provided for performing microfluidics analysis, which apparatus includes a housing comprising a plurality of fluid lines; each of the fluid lines includes an inlet for receiving a fluid from a fluid pump and a plurality of control lines in communication with the fluid lines, each of the control lines including an inlet for
10 receiving a control fluid; the apparatus also includes a microfluidics device having a plurality of interaction cells, each of the interaction cells including an inlet for receiving one or more preparation fluids and a sample fluid, and wherein each of the interaction cells receives a different sample fluid; each of the interaction cells also includes an outlet whereby fluid may flow out of the
15 interaction cell; each of the interaction cells may also include at least one microcantilever configured to deflect in response to chemical interactions with a component of the sample fluid; and the apparatus further includes a plurality of valves in communication with the fluid lines for controlling the flow of fluid into and out of the interaction cells.

20 The apparatus may further include a plurality of microcantilevers disposed in each interaction cell, and the plurality of microcantilevers may be provided in a planar array having a plurality of fingers. In accordance with related embodiments, the control fluid is a gas. The number of the plurality of valves may be less than the number of the plurality of fluid lines. Similarly, the
25 number of the plurality of valves may be less than the number of the plurality of control lines. In accordance with further related embodiments, the apparatus is mounted on a temperature-controlled platform.

 The apparatus may also include a plurality of expansion chambers for eliminating gas from fluid entering the interaction cells, and/or a waste
30 receptacle for receiving fluid from the outlets of the interaction cells. In accordance with further related embodiments, the apparatus may also include a

reservoir for sample collection from each outlet of each interaction cell, and the sample collected in at least one of the reservoirs may be subject to further analysis. The further analysis may include gel electrophoresis, for example, the gel electrophoresis may be multi dimensional. At least one of the dimensions
5 may be polyacrylamide gel electrophoresis in the presence of a denaturing detergent. The further analysis may also include mass spectroscopy.

In accordance with another embodiment, a method is provided for identifying an analyte in a plurality of sample fluids. The method includes causing a preparation solution to flow into one or more of a plurality of
10 interaction cells, wherein each of the interaction cells includes at least one microcantilever, and the preparation fluid includes a ligand that binds to the microcantilever and has affinity for the analyte; at least one sample solution flows into the one or more interaction cells, and a deflection of the microcantilever in each interaction cell having sample solution containing the
15 analyte is detected.

In accordance with related embodiments, causing the preparation solution to flow into one or more of the plurality of interaction cells further includes causing a linker solution to flow into one or more of the interaction cells, wherein the linker solution is capable of binding the ligand to the microcantilever.
20 Causing the preparation solution to flow into one or more of the plurality of interaction cells may further include causing a wash solution to flow into one or more of the interaction cells. Additionally, causing a preparation solution to flow into one or more of the plurality of interaction cells also includes causing a receptor solution to flow into one or more of the interaction cells and/or causing
25 a buffer solution to flow into one or more of the interaction cells. In accordance with related embodiments, the method may include mounting the interaction cells on a temperature-controlled platform.

In accordance with further related embodiments, the number of sample solutions may equal the number of interaction cells. Similarly the number of
30 sample solutions may be less than the number of interaction cells. The ligand may be selected from a group consisting of a protein and a nucleic acid, and the

nucleic acid may be RNA or DNA. The protein may be may be an epitope, an enzyme, or a polypeptide, and the analyte may be selected from a group consisting of all or a portion of a nucleic acid and a protein. The analyte may also be a hormone, and the hormone may be selected from a group consisting of a steroid and a polypeptide. In another related embodiment, the ligand and the analyte are each selected from a group consisting of an antibody and an antigen.

In accordance with yet another embodiment of the invention, microfluidics device includes a housing comprising a plurality of fluid lines. Each of the fluid lines includes an inlet for receiving a fluid from a fluid pump disposed within the housing. The housing also includes a plurality of control lines in communication with the fluid lines, wherein each of the control lines includes an inlet for receiving a control fluid. The embodiment also includes a microcantilever platform having a plurality of interaction cells. Each of the interaction cells includes an inlet for receiving one or more preparation fluids and a sample fluid and an outlet whereby fluid may flow out of the interaction cell. Additionally, each of the interaction cells receives a different sample fluid. A plurality of valves is in communication with the fluid lines for controlling the flow of fluid into and out of the interaction cells.

Brief Description of the Drawings

The foregoing features of the invention will be more readily understood by reference to the following detailed description, taken with reference to the accompanying drawings, in which:

Fig. 1 is a graphical illustration showing a bottom view of an apparatus for performing microfluidic analysis in accordance with an embodiment of the invention;

Fig. 2 is a graphical illustration showing a top view of the apparatus of Fig. 1;

Fig. 3 is a graphical illustration of the embodiment of Fig. 1 showing all valves closed;

Fig. 4 is a graphical illustration of the embodiment of Fig. 1 showing a

linker solution added to a first interaction cell;

Fig. 5 is a graphical illustration of the embodiment of Fig. 1 showing a linker solution added to a second interaction cell;

Fig. 6 is a graphical illustration of the embodiment of Fig. 1 showing a
5 linker solution added to a third interaction cell;

Fig. 7 is a graphical illustration of the embodiment of Fig. 1 showing a linker solution added to a fourth interaction cell;

Fig. 8 is a graphical illustration of the embodiment of Fig. 1 showing a wash solution added to a first interaction cell;

10 Fig. 9 is a graphical illustration of the embodiment of Fig. 1 showing a ligand solution added to a first interaction cell;

Fig. 10 is a graphical illustration of the embodiment of Fig. 1 showing a buffer solution added to a first interaction cell;

Fig. 11 is a graphical illustration of the embodiment of Fig. 1 showing a
15 sample solution added to a first interaction cell;

Fig. 12 is a graphical illustration of the embodiment of Fig. 1 showing a sample solution added to a second interaction cell;

Fig. 13 is a graphical illustration of the embodiment of Fig. 1 showing a sample solution added to a third interaction cell;

20 Fig. 14 is a graphical illustration of the embodiment of Fig. 1 showing a sample solution added to a fourth interaction cell;

Fig. 15 is a graphical illustration of an apparatus for performing microfluidic analysis in accordance with another embodiment of the invention;

Fig. 16 is a graphical illustration of the embodiment of Fig. 15 showing a
25 solution added to a first interaction cell;

Fig. 17 is a graphical illustration of the embodiment of Fig. 15 showing a solution added to a second interaction cell;

Fig. 18 is a graphical illustration of the embodiment of Fig. 15 showing a solution added to a third interaction cell;

30 Fig. 19 is a graphical illustration of the embodiment of Fig. 15 showing a solution added to a fourth interaction cell; and

Fig. 20 is a schematic flow chart illustrating a fluidics system for use in accordance with a method for identifying an analyte in a plurality of sample fluids in accordance with a further embodiment of the invention.

Detailed Description of Specific Embodiments

5 Fig. 1 is a graphical illustration showing a bottom view of an apparatus for performing microfluidic analysis in accordance with an embodiment of the invention. The apparatus includes three dimensional a housing 150 having a plurality of fluid lines 141-148. The fluid lines 141-148 are disposed within the housing in at least two layers such that some fluid lines are closer to a top face of
10 the housing 155 and others are closer to a bottom face of the housing 156, shown in Fig. 2. Each of the fluid lines has an inlet 131-138 for receiving a fluid from a fluid pump or other fluid delivery apparatus. Such a fluid pump may be external to the housing 150 or it may be part of the housing so as to create a completely self-contained unit. The housing 150 also includes a plurality of control lines 111-
15 120 in communication with valves 161-170. Valves 161-166 are in communication with the fluid lines 141-148. Each of the control lines 111-120 receives a control fluid, such as a gas or other fluid, from an inlet 101-110. The fluid lines 141-148, control lines 111-120 and fluid paths (discussed below) may be about 0.5 mm in diameter. For example, the diameter of the lines and paths may range from
20 about 0.05 mm to about 0.6 mm. In accordance with further embodiments of the invention, the diameter of the lines and paths may be about 0.05 mm to about 0.2 mm; from about 0.1 mm to about 0.3 mm; and from about 0.2 mm to about 0.6 mm. Control fluid and other fluids may be provided to the apparatus through the use of a robotic device, or may be provided manually.

25 A plurality of valves 161-170 control the flow of fluid into and out of a microcantilever platform 180. In this embodiment, the valves 161-166 are two-way valves that communicate with the fluid lines 141-148. The valves 161-166 all lead to a common line or manifold 800 comprising fluid paths 801-803 and 445, 545, 645, and 745, and each valve has an input and an output. For example, valve
30 166 has an input 121 for receiving control fluid from control line 120 and an output 124 that permits fluid to flow both from fluid line 146 and fluid path 802.

In other words, valve **166** controls the output of fluid line **146** as well as the output of fluid path **802**, which runs under fluid line **146**. As shown in Fig. 2, valves **167-170** are also two way valves and each of these has a valve inlet **267-270** and a valve outlet **277-280**. In order for fluid to flow through the housing, at least one of valves **167-170** must be open.

The valves **161-170** may be pneumatic valves that are activated by the control fluid. In the embodiment of Figs. 1-14, the control fluid, when pressurized, serves to close the valves **161-170**. In Fig. 1 the control fluid has not been pressurized, thus the valves are all open, whereas in Fig. 3, the control fluid is pressurized and the valves **161-170** are closed. When the control fluid is a high density gas, such as air, the response time of the valves quickens. The number of valves in the apparatus may be less than, more than or equal to the number of fluid lines. Similarly, the number of valves may be less than, equal to or more than the number of control lines.

The microcantilever platform **180** is disposed in the housing **150** and includes a plurality of interaction cells **181-184**. Each of the interaction cells **181-184** has an inlet **171-174** for receiving one or more preparation fluids and a sample fluid and an outlet, **271-274** as shown in Fig. 2, for releasing fluid from the cell through output lines **175-178**. The interaction cells may be about 4 mm in diameter. For example, the diameter of the interaction cells may range from about 0.5 mm to about 6mm. In accordance with further embodiments of the invention, the diameter of the interaction cells may range from about 0.5 mm to about 2.5 mm; from about 1 mm to about 3 mm; from about 2 mm to about 5 mm; or from about 3mm to about 6 mm.

In accordance with an embodiment of the invention, the microcantilever platform **180** is a micro-mechanical system wherein each of the interaction cells includes at least one microcantilever configured to deflect in response to interactions with a chemical component of the sample fluid. Alternatively, each of the interaction cells **181-184** may include a plurality of microcantilevers provided in a planar array of fingers.

As used herein, the term "microcantilever" is a structural term that refers

to a flexible beam that may be bar-shaped, V-shaped, or have other shapes, depending on its application. One end of the microcantilever may be fixed on a supporting base with another end standing freely. Microcantilevers are usually of microscopic dimensions, for example, they can be about $50\mu\text{m}$ to about $750\mu\text{m}$ in length. In accordance with an embodiment of the invention, the microcantilevers are preferably $200\mu\text{m}$ to $700\mu\text{m}$ in length, more preferably $250\mu\text{m}$ to $600\mu\text{m}$ in length, and most preferably $300\mu\text{m}$ to $500\mu\text{m}$ in length. Further, the width can be, for example, about $50\mu\text{m}$ to about $300\mu\text{m}$. Each microcantilever may be from about $0.5\mu\text{m}$ to about $4.0\mu\text{m}$ thick. Silicon and silicon nitride are the most common molecules used to fabricate microcantilevers. However, other molecules may be used for making microcantilevers, including piezoelectric molecules, plastic molecules and various metals.

In accordance with embodiments of the invention, the microcantilevers can be manufactured from ceramics, silicon, silicon nitride, other silicon compounds, metal compounds, gallium arsenide, germanium, germanium dioxide, zinc oxide, diamond, quartz, palladium, tantalum pentoxide, and plastic polymers. Plastics can include: polystyrene, polyimide, epoxy, polynorbornene, polycyclobutene, polymethyl methacrylate, polycarbonate, polyvinylidene fluoride, polytetrafluoroethylene, polyphenylene ether, polyethylene terephthalate, polyethylene naphthalate, polypyrrole, and polythiophene. Microcantilevers that are custom fabricated may be obtained from, for example, Diffraction Ltd., Waitsfield, VT. Further, U.S. patent numbers 6,096,559 issued August 1, 2000, and 6,050,722 issued April 18, 2000, describe fabrication of a microcantilever, including use of material such as ceramics, plastic polymers, quartz, silicon nitride, silicon, silicon oxide, aluminum oxide, tantalum pentoxide, germanium, germanium dioxide, gallium arsenide, zinc oxide, and silicon compounds.

Microcantilevers that can be employed in accordance with the invention may have a compound immobilized on the surface of a free end to detect and screen receptor/ligand interactions, antibody/antigen interactions and nucleic acid interactions as is disclosed in U.S. patent number 5,992,226, issued on

November 30, 1999. Microcantilevers can be used to detect enzyme activities directed against a substrate located on a surface of the microcantilever.

Deflection may be measured using either of optical or piezoelectric methods.

Further, the microcantilevers of the embodiments of the invention can measure

5 concentrations using electrical methods to detect phase difference signals that can be matched with natural resonant frequencies as shown in U.S. patent number 6,041,642, issued March 28, 2000. Determining a concentration of a target species using a change in resonant properties of a microcantilever on which a known molecule is disposed, for example, a biomolecule selected from DNA, RNA, and
10 protein, is described in U.S. patent number 5,763,768.

In accordance with embodiments of this invention, a method and apparatus for detecting and measuring physical and chemical parameters in a sample media may use micromechanical potentiometric sensors as disclosed in U.S. patent number 6,016,686, issued January 25, 2000. Chemical detection of a
15 chemical analyte is described in U.S. patent number 5,923,421, issued July 13, 1999. Further, magnetic and electrical monitoring of radioimmune assays, using antibodies specific for target species which cause microcantilever deflection (e.g., magnetic beads binding the target to the microcantilever, as described in U.S. patent number 5,807,758, issued September 15, 1998) would be consistent with
20 embodiments of the invention.

The term "first surface" as used herein refers to that geometric surface of a microcantilever designed to receive and bind to a ligand and further to an analyte. One or more coatings can be deposited upon this first surface. The term "second surface" refers to the area of the opposite side of the microcantilever that
25 is designed not to receive the ligand or bind to the analyte. As the second surface is generally not coated, it is generally comprised of the material from which the microcantilever or microcantilever array is fabricated, prior to any coating procedure applied to the first surface. Alternatively, it may be coated with a material different from the first surface's coating.

30 Coating of micromechanical sensors with various interactive molecules is described in U.S. patent number 6,118,124, issued September 12, 2000. A coating

material is deposited on a microcantilever by depositing a metal which may be selected from at least one of the group consisting of aluminum, copper, gold, chromium, titanium, silver, and mercury. Further, a plurality of metals may be deposited on a microcantilever by depositing, for example, a first layer of chromium and a second layer of gold, or a first layer of titanium and a second layer of gold. Coatings may be amalgams or alloys comprising a plurality of metals.

In accordance with embodiments of the invention, a first surface of a microcantilever can be fabricated to have an intermediate layer, for example, sandwiched between the first surface comprising for example, gold, and the second surface, comprising for example silicon nitride. The intermediate layer may be an alloy comprising a plurality of metals. For example, the intermediate layer may be an amalgam comprising mercury with at least one of chromium, silver, and titanium.

A microcantilever may deflect or bend from a first position to at least a second position due to differential stress on a first surface of the microcantilever in comparison to a second surface. That is, a microcantilever may deflect in response to the change in surface stress resulting from exposure of the microcantilever to a component of a particular environment. A microcantilever may also deflect in response to a change in the environment. A change in the environment may occur as the result of adding a sample having or lacking an analyte, having a higher or lower analyte concentration, adding or omitting a specific co-factor of an analyte, having a higher or lower concentration of the co-factor, having or lacking a specific inhibitor of an analyte, or having a higher or lower concentration of an inhibitor. Further, a sample may be diluted or concentrated and a solution may experience a change in temperature, pH, conductivity or viscosity prior to, during or after exposure to a microcantilever.

When one end of a microcantilever is fixed to a supporting base as described above, deflection is measured by measuring a distance the distal end of the microcantilever (i.e., the end distal to the end fixed to the supporting base) has moved. The distal end may move from a first position to a second position.

In the first position, the biomaterial on the first surface of the microcantilever has not yet bound to or reacted with the analyte. In the second position, the biomaterial on the microcantilever has bound to or has reacted with the analyte in the environment.

5 A "deflection characteristic", as used herein, is a pattern of deflection of a microcantilever that is reproducible in extent of distance traveled, for example as measured in nm, and frequency per unit time. The deflection characteristic can distinguish specific conditions of ligand and analyte, and further reaction conditions such as temperature, concentration, ionic strength, presence of cation
10 or other co-factors, preservatives, and other conditions well known to one of the chemical arts. The deflection under these conditions thereby can become a signature for the specific reaction. A deflection characteristic is calculated from a measurement of movement of the microcantilever upon addition of a sample, or measurement of movement as a function of concentration of an analyte, a ligand,
15 an inhibitor, or a co-factor. A deflection characteristic may also be calculated as a function of pH, or of temperature, and the like.

Each of the interaction cells **181-184** may receive a different sample fluid as will be discussed in more detail below. A microprocessor can be included in an apparatus or a method, such that an integrated circuit containing the arithmetic,
20 logic, and control circuitry required to interpret and execute instructions from a computer program may be employed to control activation of the the valves. Further, microprocessor components of the measuring devices may reside in an apparatus for detection of microcantilever deflection.

The apparatus may also include a plurality of expansion chambers **151-154**
25 for eliminating gas from fluids entering the interaction chamber **181-184**, and a waste line **190** with a waste outlet **191** for releasing waste from the interaction cells **181-184** into a waste receptacle (item **909** in Fig. 20). Further, each interaction cell **181-184** may be in fluid communication with its own waste receptacle or with a reservoir for collecting the contents of the interaction cell in
30 order to perform further analysis on what is contained in the reservoir. Further analysis may include gel electrophoresis, and the gel electrophoresis may be

multi-dimensional. Additionally, at least one of the dimensions may be polyacrylamide gel electrophoresis in the presence of a denaturing detergent. Further analysis may also include mass spectroscopy.

The apparatus of Figs. 1 and 2 may be a card or cartridge consisting of about 17 layers of one more plastic polymers. Such cards and cartridges may be custom manufactured, for example, by Micronics, Inc. of Redmond, Washington. These cards or cartridges may be mounted in a manifold that receives fluid pump lines or fluid from other fluid delivery devices. Similarly, the pumps may be part of the card as mentioned above. The apparatus may also be mounted on a temperature-controlled platform. The apparatus may be used to identify a particular molecule in one or more sample fluids, as is shown in Figs. 3-14.

In Fig. 3, a control fluid, in this case a gas such as air, is pressurized in the control lines 111-120 through the inlets 101-110 to close the valves 161-170. When all the valves have been closed, fluid cannot flow into or out of the interaction cells. Thus, all of the valves are initially closed (by pressurizing a control fluid in the control lines) and then opened (by de-pressurizing the control fluid) to allow liquid to flow into appropriate interaction cells. This is done so that preparation fluids, such as linker, buffer, ligand solutions, and sample solutions containing an analyte may be input to the interaction cells 181-184 in a discriminatory manner. For example, a buffer solution may be input to all of the cells or to a subset of the cells, for example, to three of the cells, two of the cells or only to one of the cells. Similarly, a different sample solution may be input to each of the cells, or to a subset of the cells.

Fig. 4 shows a linker solution being added to a first interaction cell. The term "linker solution" may include the following compounds: dithiobis(succinimidyl-undecanoate) (DSU), which can be purchased from Pierce Endogen, Inc. (Rockford, IL); long chain succinimido-6[3-(2-pyridyldithio)-propionamido] hexanoate (LCSPDP), which contains pyridyldithio and NHS ester reactive groups that react with sulfhydryl and amino groups and can be purchased from Pierce; succinimidyl-6[3-(2-pyridyldithio)-propionamido] hexanoate (SPDP), which contains pyridyldithio and NHS ester reactive groups

that react with sulfhydryl and amino groups and can be purchased from Pierce; and m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), which contains NHS ester and maleimide reactive groups that react with amino and sulfhydryl groups, and can be purchased from Pierce.

5 To add linker to the first interaction cell, inlets **109** and **106** do not receive the control gas, thus no control gas is input to control lines **119** and **116**, and valves **165** and **170** are opened. The linker solution flows from a fluid pump or other fluid delivery device to inlet **135** into fluid line **145**. Since valve **165** is open, the fluid may then flow through fluid path **445** into fluid path **446**, and into
10 expansion chamber **154**. Gas may optionally be eliminated from the linker solution in the expansion chamber **154**, and the linker solution flows through fluid path **447** into interaction cell **184** via inlet **174**. Any outflow of fluid from the interaction cell **184** will flow into output line **178**, and because valve **170** is open, the outflow will be stored in a waste receptacle (or in a reservoir for
15 collection) via fluid waste line **190** and waste outlet **191**.

 Fig. 5 illustrates how the linker solution may be added to the second interaction cell, while keeping all other interaction cells isolated, by pressurizing the control fluid in all control lines except control lines **115** and **119**, thus opening valves **165** and **169**. As above, the linker solution flows from a fluid pump to
20 inlet **135** into fluid line **145** and then through fluid paths **445** and **545**. Note that the control lines **111**, **112**, **117**, and **118** intersect fluid lines **141**, **142**, **143** and **144** respectively at a point above valves **161-164**. Consequently, fluid may flow from fluid path **445** to **545** in a relatively unrestricted manner. At this point the fluid will flow into fluid path **546**, and then into expansion chamber **153**. Gas is
25 eliminated from the linker solution in the expansion chamber **153**, and the linker solution flows through fluid path **547** into interaction cell **183** via inlet **173**. Any outflow of fluid from the interaction cell **183** will flow into output line **177**, and because valve **169** is open, the outflow will be stored in a waste receptacle via fluid waste line **190** and waste outlet **191**.

30 Figs. 6 and 7 show the linker solution being added to interaction cells **182**

and **181** respectively. In accordance with this embodiment, each interaction cell **182** and **181** will receive the linker solution while all other cells are isolated in the manner described above with respect to Figs. 4 and 5. To add the linker solution to interaction cell **182**, control fluid will not be pressurized in control lines **114** and **119**, causing valves **165** and **168** to open. Linker solution will flow from a fluid pump to inlet **135** into fluid line **145** and then through fluid paths **445**, **545**, and **645**. The fluid will then flow into fluid path **646**, and into expansion chamber **152**. Gas will be eliminated from the linker solution in the expansion chamber **152**, and the linker solution will flow through fluid path **647** into interaction cell **182** via inlet **172**. Outflow of fluid from the interaction cell **182** will flow into output line **176**, and because valve **168** is open, the outflow will be stored in a waste receptacle via fluid waste line **190** and waste outlet **191**.

To add the linker solution to interaction cell **181**, control fluid is not pressurized in control lines **113** and **119**, causing valves **165** and **167** to open. Linker solution will flow from a fluid pump to inlet **135** into fluid line **145** and then through fluid paths **445**, **545**, **645**, and **745**. The fluid will then flow into fluid path **746**, and into expansion chamber **151**. Gas will be eliminated from the linker solution in the expansion chamber **151**, and the linker solution will flow through fluid path **747** into interaction cell **181** via inlet **171**. Outflow of fluid from the interaction cell **181** will flow into output line **175**, and because valve **167** is open, the outflow will be stored in a waste receptacle via fluid waste line **190** and waste outlet **191**.

The linker solution may be added to a subset of the plurality of interaction cells, or to all of the interaction cells, illustrated here for exemplary purposes only as four of the cells **181**, **182**, **183** and **184**, by opening valve **165** with valves **167**, **168**, **169** and **170** simultaneously. Similarly, any subset of interaction cells may receive linker solution simultaneously by opening valve **165** and the valves that correspond to the interaction cells to be filled. Further, waste line **190** may lead to a plurality of reservoirs, and the outflow from the interaction cells may be stored in respective reservoirs for further analysis. Valves may be provided to

insure that outflow from each interaction cell is stored in its corresponding reservoir. Alternatively, reservoir lines and outlets may be provided for each interaction cell, rather than one line and outlet (such as waste line 190 and outlet 191).

Fig. 8 is a graphical illustration of the embodiment of Fig. 1 showing a wash solution added to a first interaction cell. The wash solution will flow from a fluid pump or other fluid delivery device to fluid line 148 via inlet 138. In accordance with this embodiment, no control line is in direct communication with fluid line 148 (though such a control line could be provided) thus, only control line 116 is de-pressurized. Valve 170 is opened, allowing the wash solution to flow into fluid path 445 via fluid paths 801-803. From this point, the wash process continues as described above with respect to the linker solution and Fig. 4-7, to provide each interaction cell with the wash solution.

Fig. 9 is a graphical illustration of the embodiment of Fig. 1 showing a ligand solution, for example, an antibody solution, added to a first interaction cell. The ligand may react chemically with previously applied linker molecules. The ligand solution flows from a fluid pump or other fluid delivery device to fluid line 146 via inlet 136. Control lines 116 and 110 are de-pressurized and valves 170 and 166 are opened, allowing the ligand solution to flow into fluid path 445 via fluid path 803. From this point, the ligand solution proceeds through the apparatus as described above with respect to the linker and wash solutions to provide each interaction cell with the ligand solution.

Fig. 10 is a graphical illustration of the embodiment of Fig. 1 showing a buffer solution added to a first interaction cell. The buffer solution flows from a fluid pump or other fluid delivery device to fluid line 147 via inlet 137. As was the case with the wash solution, no control line is in direct communication with fluid line 147 (again, such a control line could be provided), thus only control line 116 is de-pressurized. Valve 170 is opened, allowing the buffer solution to flow into fluid path 445 via fluid paths 802-803. From this point, the buffer solution proceeds to each interaction cell in accordance with the embodiments of Figs. 4-7. A wash process may follow the addition of the buffer solution to each cell and

will proceed as described above with respect to Fig. 8.

In Fig. 11, a first sample solution having an analyte, or a control solution, is added to the first interaction cell. The first sample solution flows from a fluid pump or other fluid delivery device to fluid line 144 via inlet 134. Control lines 116 and 118 are de-pressurized and valves 170 and 164 are opened, allowing the first sample solution to flow into fluid path 446 and into expansion chamber 154. From this point, the first sample solution proceeds to the interaction cell 184 via fluid path 447 and inlet 174. Outflow of the first sample solution from the interaction cell 184 will flow into output line 178, and the outflow will be stored in a waste receptacle (or reservoir for collection) via waste line 190 (or reservoir line) and waste outlet (or reservoir outlet) 191.

Figs 12 is a graphical illustration showing that the second interaction cell may be provided with a second sample solution. To provide interaction cell 183 with the second sample solution, control lines 115 and 117 are de-pressurized, valves 169 and 163 are opened and the second sample solution flows from a fluid pump to fluid line 143 via inlet 133. The second sample solution will flow into fluid path 546 and into expansion chamber 153. The second sample solution proceeds to the interaction cell 183 via fluid path 547 and inlet 173. As above, outflow of the second sample solution from the interaction cell 183 will flow into output line 177, and the outflow will be stored in a waste receptacle (or reservoir for collection) via waste line 190 and waste outlet 191.

Fig. 13 illustrates a way to provide the third interaction cell 182 with a third sample solution, control lines 112 and 114 are de-pressurized, valves 162 and 166 are opened and the third sample solution flows from a fluid pump to fluid line 142 via inlet 132. The third sample solution will flow into fluid path 646 and into expansion chamber 152 and gas will be removed from the solution. The third sample solution proceeds to the interaction cell 182 via fluid path 647 and inlet 172. Outflow of the third sample solution from the interaction cell 182 will flow into output line 176, and the outflow will be stored in a waste receptacle (or reservoir for collection) via waste line 190 and waste outlet 191.

Fig. 14 illustrates a way to provide the fourth interaction cell **181** with a fourth sample solution. Here, control lines **111** and **113** are de-pressurized, valves **161** and **167** are opened and the fourth sample solution flows from a fluid pump to fluid line **141** via inlet **131**. The fourth sample solution will flow into fluid path **746** and into expansion chamber **151**, and gas will be removed from the solution. The third sample solution proceeds to the interaction cell **181** via fluid path **747** and inlet **171**. Outflow of the third sample solution from the interaction cell **181** will flow into output line **175**, and the outflow will be stored in a waste receptacle (or reservoir for collection) via waste line **190** and waste outlet **191**.

Each of the interaction cells includes at least one microcantilever, or an array of microcantilevers, configured to deflect in response to chemical interactions with a component of the sample fluid. In a particular embodiment of the invention, a planar array of microcantilever fingers is disposed in each interaction cell such that one or more microcantilever finger deflects with respect to the plane of the array in response to a reaction with a molecular component of the sample solution.

Fig. 15 is a graphical illustration showing an apparatus for performing microfluidic analysis in which the valves of the apparatus are normally closed in accordance with another embodiment of the invention. Unlike the embodiment of Fig. 1, in Fig. 15, all of the valves **1051-1058** and **1061-1064** are closed under normal atmospheric pressure (when only air is in the lines). This configuration reduces the duty cycle of the electrical components of the system and minimizes the amount of current needed to drive the system. However, whether the valves are open or closed under normal atmospheric conditions is purely arbitrary and each of the embodiments of Fig. 1 and Fig. 15 may operate either way with respect to the configuration of lines and valves. Additionally, in accordance with the embodiment of Fig. 15, the fluid lines **1011-1019** and fluid inlets **1001-1008** are at the top of the figure, rather than at the bottom as in Fig. 1.

The apparatus includes a three-dimensional housing **1000** having a plurality of fluid lines **1011-1019**. Each of the fluid lines **1011-1018** has an inlet **1001-1008** for receiving a fluid from a fluid pump or other fluid delivery

apparatus. The housing **1000** also includes a plurality of control lines **1031-1042** in communication with the fluid lines **1011-1019**. Each of the control lines **1031-1042** receives a control fluid from an inlet **1071-1082**. The fluid lines **1011-1019**, control lines **1031-1042** and fluid paths of this embodiment may be dimensioned in a manner similar to the fluid lines, control lines, and fluid paths described with respect to Fig. 1. Here again, control fluid and other fluids may be provided to the apparatus through the use of a robotic device, or may be provided manually.

The plurality of valves **1051-1058** and **1061-1064** control the flow of fluid into and out of a microcantilever platform **1020**. The valves may be two-way valves that function as three-way valves as described above with respect to the embodiment of Fig. 1. Thus, each valve has an inlet and an outlet. For example, valve **1051** has a valve inlet **1082** for receiving the control fluid from control line **1031**, a valve outlet **1083** for transmitting fluid from fluid line **1012** to the manifold **1100**. The valves **1051-1058** and **1061-1064** are activated (in this case opened) by the control fluid. As above, when the control fluid is a high density gas the response time of the valves quickens. The valves may be activated or deactivated under control of a computer program resident on a microprocessor. Further, the number valves in the apparatus may be less than, more than or equal to the number of fluid lines. Similarly, the number of valves may be less than, equal to or more than the number of control lines.

The microcantilever platform **1020** is disposed in the housing **1000** and includes a plurality of interaction cells **1021-1024**. Each of the interaction cells **1021-1024** has an inlet, such as **1025**, for receiving one or more preparation fluids and a sample fluid and an outlet, such as **1026**, for releasing fluid from the cell through output lines **1095-1098**.

The apparatus of Fig. 15 may further include a waste line **1200** with a waste outlet **1009** for releasing waste from the interaction cells **1021-1024** into a waste receptacle. As was the case with the embodiment of Fig. 1, each interaction cell **1021-1024** may be in fluid communication with its own waste receptacle or with a reservoir for collecting the contents of the interaction cell in order to perform further analysis on what is contained in the reservoir.

As was the case with the apparatus of Fig. 1, the embodiment of Fig. 15 may be in the form of a card or cartridge comprising one more plastic polymers. Preparation fluids, such as linker, buffer, ligand solutions, and sample solutions may be input to the interaction cells **1021-1024** in a discriminatory manner. A
5 buffer solution may be input to all of the cells or to a subset of the cells, for example, to three of the cells, two of the cells or only to one of the cells. Similarly, a different sample solution may be input to each of the cells, or to a subset of the cells.

Fig. 16 shows a solution being added to a first interaction cell. To
10 accomplish this, inlets **1081** and **1079** receive the control gas, thus control gas is input to control lines **1041** and **1039** respectively, and valves **1057** and **1064** are opened. The solution flows from a fluid pump or other fluid delivery device to inlet **1008** into fluid line **1018**. Since valve **1057** is open, the fluid may then flow through fluid path **1300** into fluid path **1400**, and into interaction cell **1024** via
15 inlet **1425**. Any outflow of fluid from the interaction cell **1024** will flow into output line **1098**, and because valve **1064** is open, the outflow will be stored in a waste receptacle (or in a reservoir for collection) via fluid waste line **1200** and waste outlet **1009**.

The solution may be added to a subset of the plurality of interaction cells,
20 or to all of the interaction cells, illustrated here for exemplary purposes only as four cells. Similarly, any subset of interaction cells may receive a solution simultaneously by opening the valves that correspond to the appropriate interaction cells to be filled (as will be evident from the descriptions Figs. 16-20). Further, the waste line **1200** may lead to a plurality of reservoirs, and the outflow
25 from the interaction cells may be stored in respective reservoirs for further analysis. Valves may be provided to insure that outflow from each interaction cell is stored in its corresponding reservoir. Alternatively, reservoir lines and outlets may be provided for each interaction cell, rather than one line and outlet.

Fig. 17 is a graphical illustration of the embodiment of Fig. 15 showing a
30 solution added to a second interaction cell. Here, inlets **1080** and **1078** receive the control gas, thus control gas is input to control lines **1040** and **1038** respectively,

and valves 1056 and 1063 are opened. The solution flows to inlet 1007 into fluid line 1017. Since valve 1056 is open, the fluid may then flow through fluid path 1500 into fluid path 1600, and into interaction cell 1023 via inlet 1525. Any outflow of fluid from the interaction cell 1023 will flow into output line 1097, and because valve 1063 is open, the outflow will be stored in a waste receptacle or reservoir via fluid waste line 1200 and waste outlet 1009.

Fig. 18 is a graphical illustration of the embodiment of Fig. 15 showing a solution added to a third interaction cell. Inlets 1075 and 1077 receive the control gas, and control gas is input to control lines 1035 and 1037 respectively. Valves 1055 and 1062 are opened. The solution flows from a fluid pump or other fluid delivery device to inlet 1006 into fluid line 1016. Since valve 1055 is open, the fluid may then flow through fluid path 1700 into fluid path 1800, and into interaction cell 1022 via inlet 1725. Again, outflow of fluid from the interaction cell 1022 will flow into output line 1096, and because valve 1062 is open, the outflow will be stored via fluid waste line 1200 and waste outlet 1009.

Fig. 19 is a graphical illustration of the embodiment of Fig. 15 showing a solution added to a fourth interaction cell. Inlets 1074 and 1076 receive the control gas, which is input to control lines 1034 and 1036 respectively. Valves 1054 and 1061 are opened, and the solution flows from to inlet 1005 into fluid line 1015. Since valve 1054 is open, the fluid may then flow through fluid path 1900 into fluid path 2000, and into interaction cell 1021 via inlet 1225. Any outflow of fluid from the interaction cell 1021 will flow into output line 1095, and because valve 1061 is open, the outflow will be stored in a waste receptacle or reservoir via fluid waste line 1200 and waste outlet 1009.

Fig. 20 is a schematic flow chart illustrating fluidics system for use in accordance with a method for identifying an analyte in a plurality of sample fluids in accordance with a further embodiment of the invention. In accordance with this embodiment, one or more preparation solutions 901-904 are input into one or more of a plurality of interaction cells. At least one of the preparation fluids includes a ligand that has affinity for the analyte. Each interaction cell

includes at least one microcantilever such that the ligand binds to the microcantilever. At least one sample solution 905-908 is input into one or more of the interaction cells, and a deflection of the microcantilever is detected in each sample solution containing the analyte. In one embodiment, the device may
5 be mounted in a manifold and/or on a temperature-controlled platform. Outflow from the interaction cells 910-913 may be stored in a waste receptacle 909 or in a reservoir for further analysis as described above.

One of the preparation fluids may be a solution of a linker 901 capable of covalently linking the ligand, here defined as the material affixed to a surface of
10 the microcantilever, to the microcantilever. Another preparation fluid may be a wash solution 902, and the wash solution may be input to one or a plurality of the interaction cells one or more times. Yet another preparation fluid may be a ligand or a "receptor" solution 903, i.e., a biological macromolecule known to have affinity for a specific binding portion, or a ligand for a class of analytes. The
15 receptor can also be a ligand for an analyte, the presence and/or amount of which is to be detected in one or in a series of sample. Another preparation fluid may be a buffer solution 904. The number of sample solutions may equal the number of interaction cells or the number of sample solutions may be less than the number of interaction cells.

The ligand may be a biomaterial, for example, a protein such as an enzyme or a synthetic polypeptide, or it can be a nucleic acid such as RNA or DNA. A biomaterial that is a macromolecule may comprise all or a portion of a nucleic acid or a protein. The protein or polypeptide may comprise an epitope, an antibody, an antibody fragment, an enzyme, or any other embodiment of a
20 molecule containing peptide bonds. The analyte to be detected or quantified in a sample may be a biomaterial such as a macromolecule, or an organic or inorganic small molecule. Similarly, the analyte may be hormone, for example, the hormone may be a steroid for example, a sex steroid or a glucocorticoid, or a polypeptide hormone such as a cytokine. Either of the ligand or the analyte may
25 comprise all or a portion of an antibody or an antigenic material, or all or a
30 portion of an enzyme.

Examples and methods for the use of the apparatus of the invention are shown in Table 1. In Example 1, the apparatuses of Figs. 3-19 is used to demonstrate a movement or deflection of a plurality of microcantilevers in a microcantilever array when a sample solution contains an analyte, such as a particular chemical or biological component, capable of binding to or interacting with a ligand affixed to a surface of the microcantilever. Cell A can be a reaction cell that provides a positive control; deflection of microcantilevers is caused by interaction on a surface of the cantilever of components of fluids sequentially provided to cell A. Cell B can be a reference cell; for example, a control buffer known to lack the analyte, is added to this cell instead of a sample. This control can determine the extent of microcantilever deflection that occurs as a result of interactions between preparation liquids such as a linker solution and an antibody solution, or other environmental forces. Cell C can be a negative control cell, for example, which has not been exposed to linker solution. Microcantilever deflection in this cell can determine the extent of ligand binding to a microcantilever surface directly, in the absence of a cross-linking agent. Cell D can be another control cell, containing for example, bovine serum albumin instead of the biomaterial of interest, so that microcantilever deflection is a measure of non-specific binding of the analyte.

The contents of all cited references are hereby incorporated by reference herein.

Example 1

In accordance with step 1 of Example 1 as illustrated in Table 1, the cross-linking agent DSU (dithiobis(succinimidylundecanoate)) in a volume of about 50 μ l, is added to interaction cells A, B and D. DSU is a water soluble bifunctional cross-linking agent.

In step 2, as herein exemplified, all of the cells receive a wash solution in a volume of about 300 μ l per cell. In step 3, all of the cells can receive about 50 μ l of an antibody solution (such as an antibody specific for an oncogene protein such as Bcr A or Wilm's Tumor, WT-1). A buffer having a low pH is provided to interaction cells, for example, to cells A, B and D, in a volume of about 50 μ l per cell in step 4. This solution removes non-specifically bound material, i.e., those

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molecules of material which have not reacted with the cross-linking agent. Cells are washed with about 300 μ l of the wash solution in step 5. In step 6, a volume of a sample solution containing, for example, an unknown quantity of a material that can interact with the antibody of step 3, for example, about 50 μ l is provided to cells, for example, to cells A and C. A control material, e.g., bovine serum albumin is provided to cell D in a volume of about 50 μ l in step 7. Cells are washed, for example, with about 300 μ l of the wash solution in step 8.

Further in Example 1, it should be noted that the wash steps can be performed with the same solution, and that steps, for example, steps 6 and 7, can be preformed simultaneously. Further, any of the wash steps are optional in volume and timing; deflection of microcantilevers can be analyzed throughout, although measurement of deflection following steps 6 and 7 is most significant.

It is to be understood that a choice of a volume of fluid to use is merely suggested here and can be varied from the suggested amounts. Volumes for other use in the methods and apparatuses herein can be standardized within any given experiment according to a protocol to be devised by a user of ordinary skill in the art, and such alternative volumes are within the equivalents envisioned herein.

Example 2

Example 2 is an illustration of how the apparatus described herein may be used to identify a ligand in a plurality of sample solutions. Here cells A, B and C are reaction cells and cell D is used as a control cell. A volume, for example, of about 50 μ l of DSU is provided to each cell in step 1. Next in step 2, a wash solution is provided to each cell in a volume of, for example, about 300 μ l per cell. In steps 3 and 4, each cell is provided with about 50 μ l of antibody solution and buffer solution respectively, and in step 5 the cells are subjected to another wash. A first sample solution, in a volume of about 50 μ l, is then added to cell A in step 6. A second sample solution, also in volume of about 50 μ l, is added to cell B in step 7, and a third sample solution of the same volume is added to cell C in step 8. It should be noted that in accordance with the apparatus described above, the first, second and third sample solutions may be provided to cells A, B and C,

respectively, in one step. All of the cells are subjected to an optional wash process in step 9. Further, the solutions in one or more of the cells may be reused. That is, additional solutions may be added to one or more of the cells for further analysis.

5 Example 3

Example 3 illustrates how the apparatus described herein may be used to diagnose a patient simultaneously for one of a plurality of different viruses. Cells A, B and C are reaction cells and cell D is used as a control cell. A volume, for example, of about 50 μ l of DSU is provided to each cell in step 1. In step 2, a wash solution is provided to each cell in a volume of, for example, about 300 μ l per cell. In step 3 cell A is provided with about 50 μ l of a first antibody solution. In step 4 cell B is provided with about 50 μ l of a second antibody solution, and in step 5 cell C is provided with about 50 μ l of a third antibody solution. Each antibody solution can have binding determinants directed against one of the viruses for the diagnosis. A volume of about 50 μ l, of buffer solution is added to each of the cells step 6. All of the cells are then provided with about 300 μ l of a wash solution in step 7, and in step 8 a volume of about 50 μ l of a first, second, third sample solutions is provided to cells A, B, and C. The first, second and third antibody solutions may be provided to cells A, B and C, respectively, in one step. All of the cells can be subjected to an optional wash process in step 9.

Although various exemplary embodiments of the invention have been disclosed, it should be apparent to those skilled in the art that various changes and modifications can be made which will achieve some of the advantages of the invention without departing from the true scope of the invention.

Table I

Example 1

Step	Solution	Total Vol μ l (All Cells)	A Reaction	B Ref	C NSB	D BSA
1	DSU	50	+	+	0	+
2	Wash	300	+	+	+	+
3	Ab	50	+	+	+	+
4	Hbuffer	50	+	+	0	+
5	Wash	300	+	+	+	+
6	Sample	50	+	0	+	0
7	BSA	50	0	0	0	+
8	Wash	300	+	+	+	+

Example 2

Step	Solution	Total Vol μ l (All Cells)	A Reaction	B Reaction	C Reaction	D Control
1	DSU	50	+	+	+	+
2	Wash	300	+	+	+	+
3	Ab	50	+	+	+	+
4	Hbuffer	50	+	+	+	+
5	Wash	300	+	+	+	+
6	Sample 1	50	+	0	0	0
7	Sample 2	50	0	+	0	0
8	Sample 3	50	0	0	+	0
9	Wash	300	+	+	+	+

Example 3

Step	Solution	Total Vol μ l (All Cells)	A Reaction	B Reaction	C Reaction	D Control
1	DSU	50	+	+	+	+
2	Wash	300	+	+	+	+
3	Ab 1	50	+	0	0	0
4	Ab 2	50	0	+	0	0
5	Ab 3	50	0	0	+	0
6	Hbuffer	50	+	+	+	+
7	Wash	300	+	+	+	+
8	Sample	50	+	+	+	0
9	Wash	300	+	+	+	+